Supporting Information

Glass et al. 10.1073/pnas.1118448109

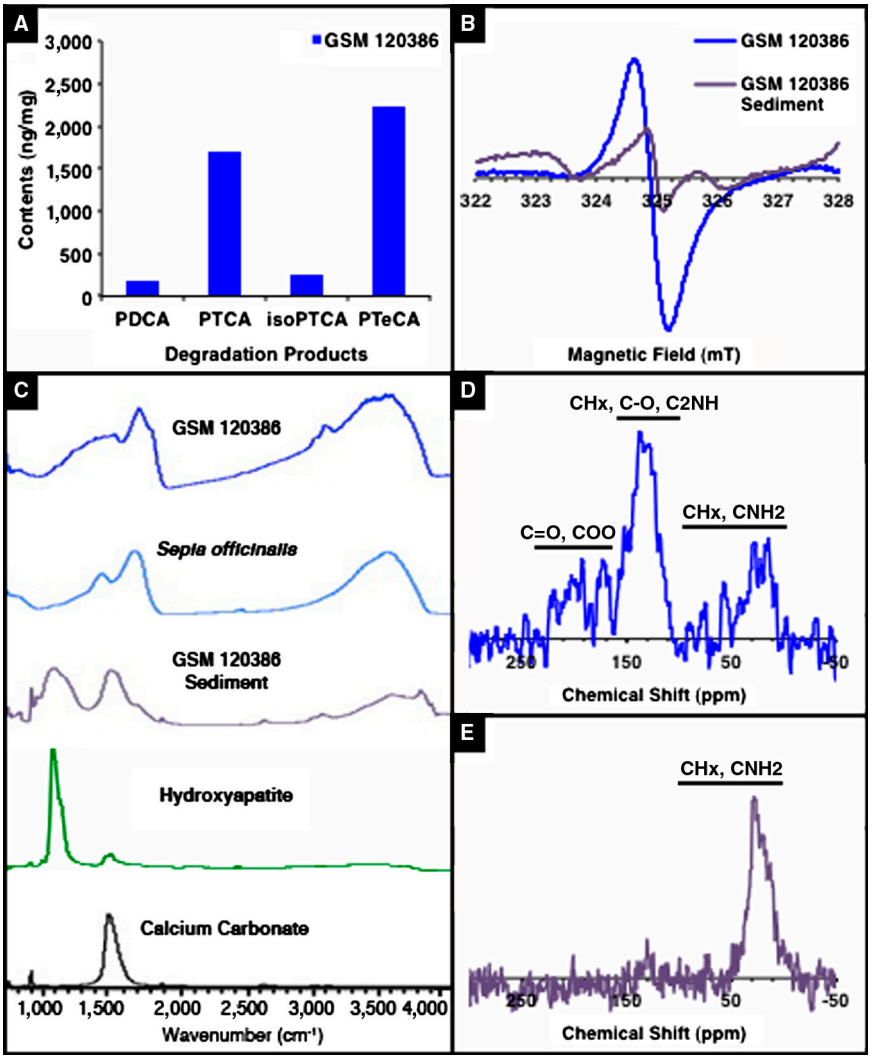

Fig. S1. (*A*) Quantification of melanin markers produced by chemical degradation of fossil specimen GSM 120386 (Table S1). Degradation of the sediment of GSM 120386 yielded 2.4 ng/mg PDCA, 3.2 ng/mg PTCA, <3.9 ng/mg isoPTCA, and <1.5 ng/mg of PTeCA. (*B*) The EPR spectra of GSM 120386 and its sediment, exhibited the linewidth and g-factor characteristic of eumelanin. The same quantity of sample pigment and sediment were used to provide a qualitative comparison of the amount of eumelanin present. (*C*) IR absorption spectra for GSM 120386, *S. officinalis* melanin, GSM 120386 sediment, and standards of hydroxyapatite and calcium carbonate. (*D–E*) ^{13}CP-MAS SSNMR spectrum of ink collected from GSM 120386 and the spectrum of the sediment from GSM 120386.

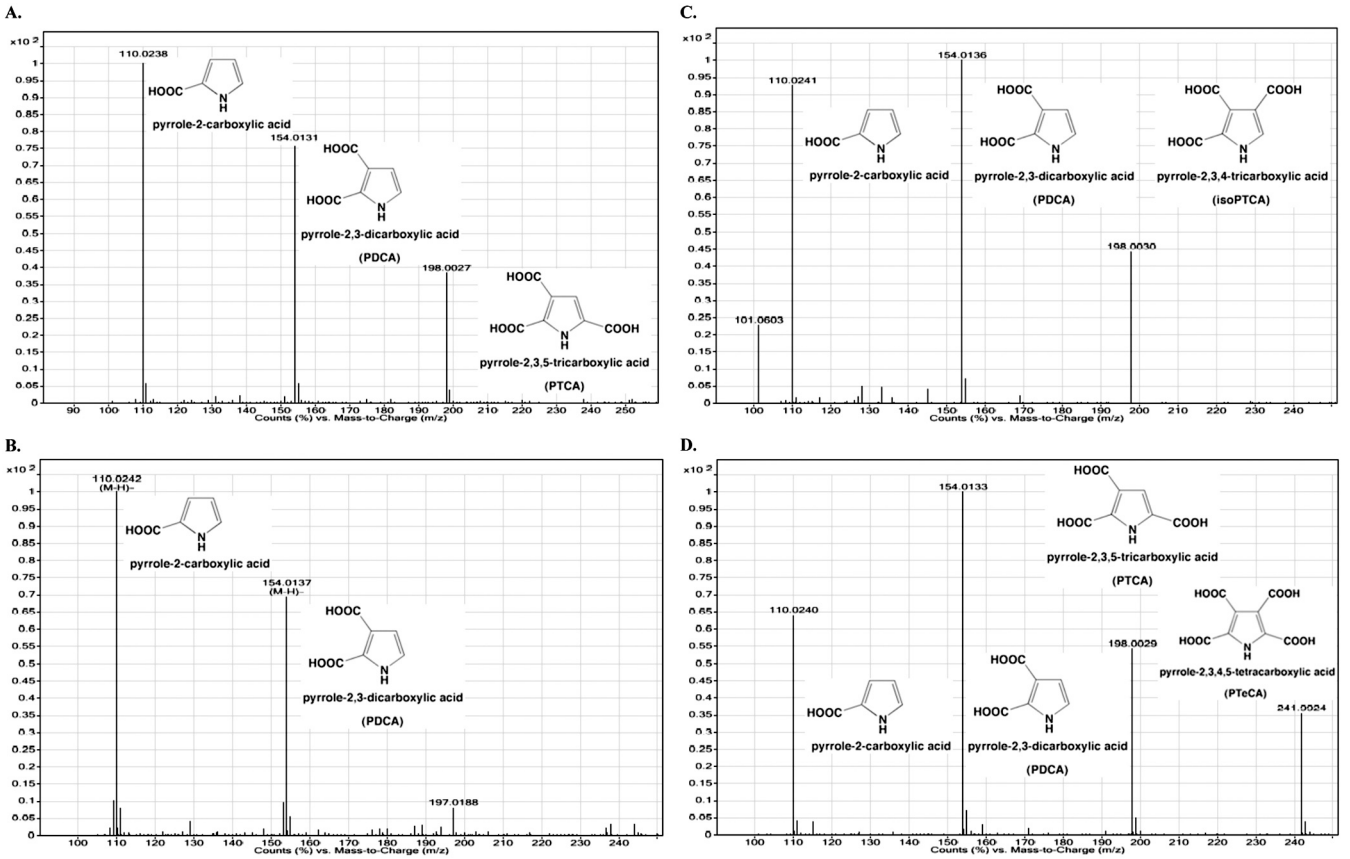

Fig. S2. Mass spectrum of the degradation products (*A*) PTCA, (*B*) PDCA, (*C*) isoPTCA, and (*D*) PTeCA from the GSM 120386 sample. Structures associated with the peaks of the fragments formed from the parent ion, the ion with the greatest mass in each of the spectrum above, are indicated.

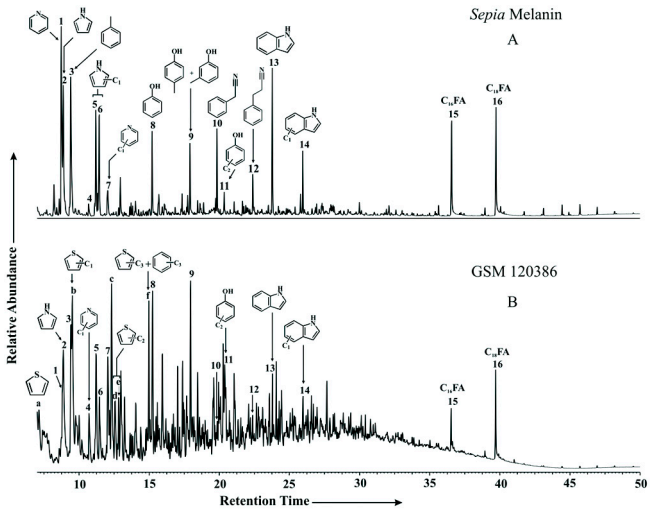

Fig. S3. Total ion chromatograms following pyrolysis at 600 °C for 20 s of (*A*) *S. officinalis* melanin and (*B*) GSM 120386. The numbers indicate major pyrolysis products of melanin: 1, pyridine; 2, pyrrole; 3, toluene; 4, C_1 pyridine; 5, C_1 pyrrole; 6, C_1 pyrrole; 7, C_1 pyridine; 8, phenol; 9, 4- and 5-methylphenol; 10, 2-phenylacetonitrile; 11, C_2 phenol; 12, 3-phenylpropanenitrile; 13, indole; 14, C_1 indole; 15, palmitic acid; 16, stearic acid. Letters indicate diagenetic products of melanin: a, thiophene; b, C_1 thiophene; c, C_2 thiophene and C_2 benzene; d, C_2 thiophene; e, C_2 thiophene; f, C_3 thiophene and C_3 benzene.

Table S1. The absorbance at 500 nm of Soluene-350 solubilized GSM 122841, GSM 120386, GSM 122841 sediment, GSM 120386 sediment, and *S. officinalis* melanin

Specimen	A500/mg	PDCA	PTCA	isoPTCA	PTeCA
GSM 122841	1.45	58	342	47	438
GSM 120386	8.13	173	1710	245	2220
GSM 122841 sediment	0.135	1.7	2.2	<2.2	<1.3
GSM 120386 sediment	0.100	2.4	3.2	<3.9	<1.5
S. officinalis melanin	5.99	704	15710	390	2090

The quantity of the alkaline hydrogen peroxide oxidation products, PDCA, PTCA, isoPTCA, and PTeCA in ng/mg for each sample.

Table S2. Mass spectral data for the degradation products derived from fossil specimen GSM 120386

	Peak	Chemical formula	Calculated *m/z*	Difference in ppm
PTCA (Fig. S2*A*)	198.0031	$C_7H_5NO_6$	198.0044	6.73
	154.0135	$C_6H_5NO_4$	154.0146	7.26
	110.0241	$C_5H_5NO_2$	110.0248	6.02
PDCA (Fig. S2*B*)	154.0137	$C_6H_5NO_4$	154.0146	5.92
	110.0242	$C_5H_5NO_2$	110.0248	4.95
isoPTCA (Fig. S2*C*)	198.0030	$C_7H_5NO_6$	198.0044	6.88
	154.0136	$C_6H_5NO_4$	154.0146	6.53
	110.0241	$C_5H_5NO_2$	110.0248	6.19
PTeCA (Fig. S2*D*)	241.9924	$C_8H_5NO_8$	241.9942	7.42
	198.0029	$C_7H_5NO_6$	198.0044	7.71
	154.0133	$C_6H_5NO_4$	154.0146	8.17
	110.0240	$C_5H_5NO_2$	110.0248	7.06

Data confirms the presence of PTCA, PDCA, isoPTCA, and PTeCA in the fossilized specimen. Each decarboxylation results in a decrease in the mass to charge ratio by 44. Mass spectra are shown in Fig. S2.

Table S3. Chemical analysis of synthetic 1:1 DHI:DHICA eumelanin before and after exposure to 100 °C for 18 d and before and after exposure to 40 °C for 70 d

Specimen	PTCA (ng/mg)	PTeCA (ng/mg)	PDCA (ng/mg)	PTeCA/PTCA
Before heating at 100 °C	27,710	3,180	1,690	0.11
After heating at 100 °C	8,720	9,220	960	1.06
Before heating at 40 °C	27,500	2,510	1,010	0.09
After heating at 40 °C	18,800	4,280	970	0.23

Table S4. Elemental analysis of the C, N, and H in synthetic DHI-melanin, DHICA-melanin, *S. officinalis* melanin, GSM 122841, GSM 120386, GSM 122841 sediment, GSM 120386 sediment, and standards of calcium carbonate and hydroxyapatite

	Elemental analysis (%)*			
Specimen	C	H	N	Molecular composition
DHI-melanin	51.52	3.45	7.80	$C_{7.70}H_{6.14}NO_{4.18}$
DHICA-melanin	47.43	3.57	6.42	$C_{8.60}H_{7.72}NO_{5.80}$
S. officinalis melanin†	42.87	2.72	6.57	$C_{7.61}H_{5.75}NO_{6.39}$
GSM 122841‡	16.87	1.69	1.37	$C_{14.37}H_{17.15}NO_x$
GSM 120386	49.40	3.73	5.07	$C_{11.36}H_{10.22}NO_{7.22}$
GSM 122841 sediment	14.75	2.27	0.37	—
GSM 120386 sediment	8.64	0.92	0.18	—
Calcium carbonate	11.94§	0.17	0.00	$CaCO_3$
Hydroxyapatite	1.02	0.50¶	0.00	$Ca_{10}(PO_4)_6(OH)_2$

*Averages for two determinations.
†Liu et al. (1).
‡Oxygen values are not calculated because of the low N contents.
§Calculated value for C, 12.00%.
¶Calculated value for H, 0.20%.

1. Liu Y, et al. (2005) Comparison of structural and chemical properties of black and red human hair melanosomes. *Photochem Photobiol* 81:135–144.

Table S5. X-ray photoelectron spectroscopy data for the modes and percentages of the binding interactions that carbon undergoes in *S. officinalis* melanin, and fossil specimens GSM 122841 and GSM 120386

Binding energy (eV)	*S. officinalis*	GSM 122841	GSM 120386
285.0 (CH_x, $CHNH_2$)	52 ± 1	54 ± 1	54 ± 1
286.5 (C—O, C_2NH)	29 ± 1	32 ± 3	27 ± 1
288.2 (C═O)	15 ± 3	13 ± 4	17 ± 2
289.1 (COO)	4 ± 3	1 ± 1	2 ± 1

Data is derived from multiple high-resolution carbon scans of each sample calibrated to 285.0 eV for the CH_x, CNH_2 peak. Error indicated is standard deviation (SD).

Table S6. Rock-Eval pyrolysis data for GSM 122841 and GSM 120386 sediment samples

Specimen	S1*	S2*	S3†	T_{max} (°C)	TOC (%)	MIN C (%)	HI‡	OI§
GSM 122841	0.89	82.82	3.11	411	11.94	1.26	694	26
GSM 120386	0.32	14.87	2.28	424	4.50	4.16	330	51

S1, the amount of free hydrocarbons present on the sample surface obtained during pyrolysis at 300 °C; S2, the hydrocarbons produced as the temperature increases gradually from 300 °C to 650 °C and nonvolatile organic material breaks down; S3, CO_2 produced during the pyrolysis of the nonvolatile organic material; T_{max}, the temperature at which the maximum amount of hydrocarbon is released from nonvolatile organic material; TOC, the total percent organic content of a sample; MIN C, the percent carbon content from carbonate-containing minerals; HI, the result of $(100 \times S2)/TOC$; and OI, $(100 \times S3)/TOC$. Note a higher T_{max} is indicative of a more mature sample and a greater alteration of the original organic material in that sample.

*Units of mg CH/g of fossil sample.

†Units of mg CO_2/g of fossil sample.

‡Units of mg CH/g TOC where TOC refers to the total organic content.

§Units of mg CO_2/g TOC.